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MULTIPLEXED PATHOGENICITY ISLAND DETECTION OF BIOWARFARE AGENTS, ENVIRONMENTAL PATHOGENS, AND FOOD-BORNE PATHOGENS

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ABSTRACT

The Institute for Advanced Technology (IAT) at The University of Texas at Austin (UT Austin) and UT faculty have joined together with Radix BioSolutions, Ltd. (Georgetown, TX) to transition technologies developed at UT Austin. The long-term objective of this project is to provide a fieldable system composed of a sampler and pre-sample preparation module and a transduction platform. The envisioned system combines pathogenicity island sequences with a multiplexed assay platform to develop a rapid, extensive infectious pathogen sensor. This sensor is based upon the Luminex xMAP™ System, a multiplexed assay platform that combines high sample throughput (up to 600 samples/hour) with high information content per sample (up to 100 parameters tested simultaneously per sample). Pathogenicity islands are DNA sequences that directly relate the infectious properties of an organism to its host. By quickly screening for multiple pathogenicity island sequences, end-users will have the capability to detect the first signs of a bioattack without requiring screening for a particular organism. Once a pathogenicity "fingerprint" is identified, an alert is transmitted to an end user, which includes military, health providers, and other government agencies.

INTRODUCTION

Recent events in the U.S. have validated the fear that terrorists will use biowarfare agents in the homeland (anthrax) or on U.S. troops abroad (Congo-Crimean Hemorrhagic fever). Present biological agent detection systems tend to rely on the detection of single, or a few, DNA sequences, antigens or antibodies. The objective for this work is to use identified pathogenicity islands to completely define the critical domains of a set of virulence genes of key pathogens, and to use this information to design and build a prototype multiplexed detection system that will rapidly screen and detect every virulent mutant of that pathogen or pathogen class, and will give no false alarms. This detection system will have hundreds of probes (multiplexed), so that false positives and false negatives will be determined immediately. The system will be rugged, lightweight, and low-cost. A side benefit of this type of multiplexed detection is that it may inform investigators if a particular pathogen is man-made, if it is a new emergent infectious disease, or the source of the strain. The enteric pathogens include a large number of related, but distinct organisms. Identification of the organisms has been based on antigenic or biochemical analysis. While

this approach is effective, it may fail to identify potential pathogens if they are antigenic variants or differ in metabolic pathways. For example, epidemics of cholera have traditionally been caused by *Vibrio cholerae* serotype O1 and antisera against the O1 antigen was used to detect epidemic strains. However, a previously unrecognized epidemic serotype, O139, that was not detected by O1 antisera emerged recently and caused a major pandemic of cholera. An alternative approach to rapid detection and identification of pathogens is to develop methods that detect gene encoding virulence factors associated with a particular syndrome rather than detecting bacteria that may or may not have these virulence factors. We have applied this approach to rapid identification of pathogens in the *E. coli/Shigella* group. These pathogens contain the same "core" sequences but have additional regions of DNA encoding virulence determinants that appear to have been inserted into the chromosomes (as pathogenicity islands and prophages) or are carried on extrachromosomal elements (plasmids). It is the particular set of virulence factors encoded by a member of this group that determines pathogenic potential. *E. coli* O157:H7, which has been associated with large outbreaks of hemorrhagic colitis and hemolytic uremic syndrome has the basic *E. coli* K-12 genome but also carries a phage encoding the shiga-like toxin, the

LEE pathogenicity island carrying the attachment and effacing loci, the *Shu* heme transport island, and plasmids encoding virulence factors. *Shigella dysenteriae*, which causes dysentery, encodes a related Shiga toxin and has the same *Shu* island, but lacks the *LEE* and has a different plasmid that confers the ability to invade and multiply within human epithelial cells (Sansonetti 1992, Wyckoff et al. 1998). *S. flexneri* lacks the Shiga toxin locus and the *Shu* island (Wyckoff et al. 1998) but carries an island encoding a different iron transport system (aerobactin) that is not found in *S. dysenteriae* and *E. coli O157:H7* (Vokes et al. 1999). By identifying the specific factors present in a member of this group, we can determine its potential to cause disease and the type of disease. This could lead to more rapid identification of the pathogen and determination of the proper course of treatment.

The Luminex xMAP™ System provides a rapid and inexpensive method to simultaneously detect the presence of antigens or specific DNA sequences. The xMAP™ System utilizes polystyrene microspheres internally dyed with two spectrally distinct fluorochromes. Using precise ratios of these fluorochromes, a suspension array is created consisting of 100 spectrally distinct microsphere sets. Each microsphere set can possess a different reactant on its surface, and thus, up to 100 different analyses can be detected simultaneously in a single reaction vessel. A third fluorochrome coupled to a reporter molecule quantifies the biomolecular interaction that has occurred at the microsphere surface. The multiplexing capabilities of the xMAP™ system provide an assay detection platform that does not require users to screen for specific organisms, but allows a more thorough screen for the presence of biowarfare, food-borne, and environmental pathogens. As new pathogenicity islands are discovered or mutations are determined, new microsphere sets carrying these sequences will be added to the current assay kit, thus seamlessly updating the kit.

PRELIMINARY RESULTS

Recent experiments have shown that the instrument is capable of detecting multiple pathogens at less than 1000 genome copies per amplified sample. An initial pathogenicity island multiplexed assay has been developed that examines three different sequences, two for pathogenicity islands and one for a common junction area insertion site. Preliminary data demonstrates a 3.5-7 fold signal to noise ratio using as little as 1µL of PCR product for detection (Figures below).

CONCLUSION

The use of pathogencity islands can be used as indiscriminant identifiers of pathogens thus providing a genomic fingerprint to identifty potential of pathogens. The use of the Luminex xMAP™ system provides for a rapid high throughput screening system which can be modified by adding additional gene sequences as need to current sequences or imunoassays. Because the detection system is based on

multiple sequences for each pathogencity factor one can essentially eliminate false positives and false negatives.

The proposed system has commercial applications in office health monitoring, hospital infectious disease detection, environmental and agricultural pathogen monitoring, and food-borne pathogen monitoring.

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